# CLINICAL PHARMACOLOGY ROUNDS

## Deficient C-oxidation of nicotine

In most people, nicotine is extensively (70% to 80%) metabolized to cotinine by C-oxidation. In a clinical trial, a 57-year-old woman was found to have the expected plasma levels of nicotine but unusually low plasma levels of cotinine both when smoking cigarettes and while receiving transdermal nicotine. To characterize her metabolism, simultaneous infusions of deuterium-labeled nicotine ($d_2$) and cotinine ($d_4$) were administered, with comparison to 20 other control smokers. The clearance of nicotine was unusually low (6.5 ml/min/kg versus 17.2 ml/min/kg), and the half-life of nicotine significantly longer (348 minutes versus 138 minutes) in the index case subject compared with the control subjects. The clearance of cotinine was normal. The index case subject converted only 9% of nicotine to cotinine, compared with 72% for the control subjects. As far as we know, this is the first person with deficient C-oxidation of nicotine to be characterized. Deficient C-oxidation of nicotine is associated with a long half-life of nicotine and deficient generation of cotinine, both of which could influence the risks and addictiveness of tobacco use in affected individuals. (CLIN PHARMACOL THER 1995;57:590-4.)

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Tobacco addiction is sustained by the pharmacologic actions of nicotine.[1] There is evidence that smokers adapt their smoking behavior to maintain desired levels of nicotine in the body.[1,2] The level of nicotine in the body is determined both by the intake of nicotine from tobacco and the rate of metabolism of nicotine by the liver. The major pathway of metabolism of nicotine is C-oxidation to cotinine (Fig. 1).[3] Typically, 70% to 80% of nicotine is converted to cotinine.[3] Cotinine, which has a much longer half-life than nicotine and is present in the blood in manyfold higher concentrations, is widely used as a biomarker of nicotine exposure.[4] Cotinine may also have pharmacologic activity that could contribute to nicotine addiction.[5]

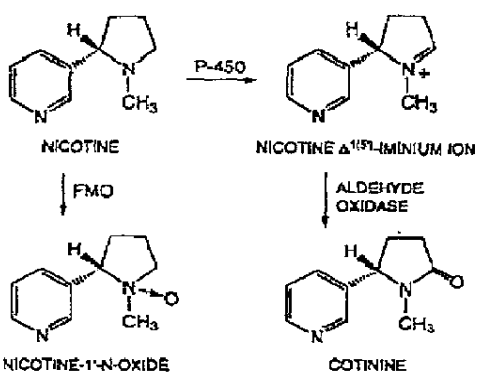


Fig. 1. Primary pathways of nicotine metabolism.

We report on a woman who had deficient C-oxidation of nicotine, resulting in little generation of cotinine and unusually slow metabolism of nicotine.

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Supported by U.S. Public Health Service grants DA02277 and DA01696 (Dr. Benowitz) and DA04986 (Dr. Sachs) from the National Institute on Drug Abuse and carried out in part in the General Clinical Research Center at San Francisco General Hospital Medical Center with support of the Division of Research Resources, National Institutes of Health (RR-00083).
Received for publication Oct. 17, 1994; accepted Dec. 22, 1994.

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0009-9236/95/$3.00 + 0 13/1/62964



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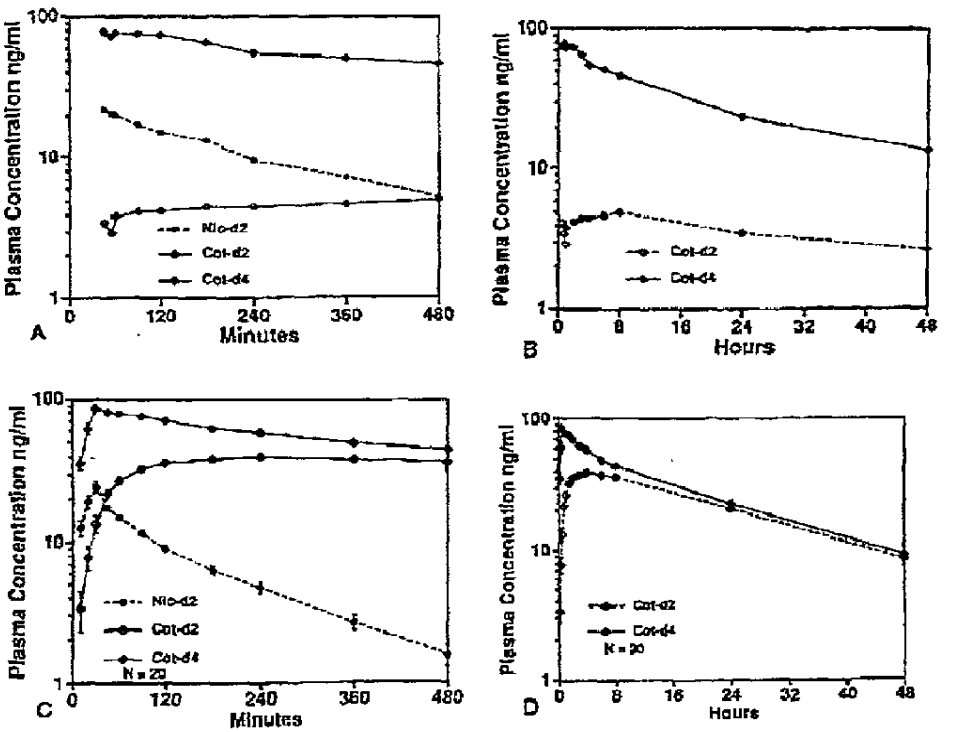


**Fig. 2.** Mean plasma concentrations of nicotine-$d_2$ (Nic-d2), cotinine-$d_2$ (Cot-d2), and cotinine-$d_4$ (Cot-d4) during and after intravenous infusions of a 50:50 mixture of nicotine-$d_2$ and cotinine-$d_4$ (2 μg base/kg/min of each for 30 minutes; beginning at time 0). A, Shows values up to 480 minutes for index case subject. B, Shows values up to 48 hours for index case subject. C, Average data for 20 control subjects up to 480 minutes. D, Average data for 20 control subjects up to 48 hours. *Bars* represent ± SEM. (C and D from Benowitz HL, Jacob P. CLIN PHARMACOL THER 1994;56:483-93. Used with permission.)

## METHODS

The metabolism of nicotine and cotinine was studied with use of a combined infusion of 3′,3′-dideuteronicotine (nicotine-$d_2$) and 2,4,5,6-tetradeuterocotinine (cotinine-$d_4$).[6] Subjects were studied at the General Clinical Research Center at San Francisco General Hospital, where infusions were performed between 8 and 9 AM after subjects fasted overnight. Subjects received a 30-minute infusion of a 50:50 mixture of nicotine-$d_2$ and cotinine-$d_4$, each at a rate of 2 μg/kg/min. Blood samples were collected at frequent intervals and urine was collected for 72 hours. Details of the synthesis of labeled compounds, the gas chromatography–mass spectrometry assays for blood concentrations, and techniques of pharmacokinetic analysis of the resultant data are described elsewhere and references cited therein.[6]

As a control group for our index case subject, 20 healthy subjects (10 men and 10 women; age range, 23 to 51 years; mean age, 36 years) who smoked an average of 23 cigarettes a day (range, 10 to 50 cigarettes a day) were studied. Written consent was obtained from each subject. The study was approved by the Committee on Human Research at the University of California, San Francisco. The data on the disposition kinetics of nicotine and cotinine and fractional conversion of nicotine to cotinine have been recently reported.[6] This report focuses on an additional indi-

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**Table I.** Disposition kinetics of nicotine and cotinine in index case subject and 20 control subjects

| | *Index case subject* | *Control subjects (mean)* | *95% CI* |
|---|---|---|---|
| Body weight (kg) | 56.2 | 70.5 | 42.6-98.4 |
| Nicotine-$d_2$ | | | |
| CL (ml/min/kg) | 6.5* | 17.2 | 6.9-27.5 |
| $CL_R$ (ml/min/kg) | 2.0 | 0.7 | 0-2.2 |
| $CL_{NR}$ (ml/min/kg) | 4.4* | 16.3 | 4.6-26.6 |
| $V_{SS}$ (L/kg) | 2.8 | 2.8 | 1.4-4.2 |
| $t_{1/2}$ (min) | 348* | 138 | 64-212 |
| Cotinine-$d_4$ | | | |
| CL (ml/min/kg) | 0.50 | 0.59 | 0.19-0.99 |
| $CL_R$ (ml/min/kg) | 0.13 | 0.08 | 0.01-0.15 |
| $CL_{NR}$ (ml/min/kg) | 0.37 | 0.51 | 0-0.93 |
| $V_{SS}$ (L/kg) | 0.93 | 0.76 | 0.37-1.16 |
| $t_{1/2}$ (min) | 1335 | 1047 | 413-1680 |
| Nicotine metabolism to cotinine | | | |
| Fc | 0.09* | 0.72 | 0.54-0.91 |
| $CL_f$ (NIC → COT) | 0.4* | 12.1 | 9.8-14.4 |

95% CI, 95% Confidence interval; CL, total plasma clearance; $CL_R$, renal clearance; $CL_{NR}$, nonrenal clearance; $V_{SS}$, steady-state volume of distribution; $t_{1/2}$, half-life; Fc, fractional conversion of nicotine to cotinine; $CL_f$, formation clearance of cotinine.
*Significantly different from control subjects.

vidual (described in more detail in the Results section). Data on renal clearance ($CL_R$), nonrenal clearance ($CL_{NR}$), and formation clearance ($CL_f$) of cotinine (metabolic clearance of nicotine to cotinine) are presented for this individual and for the control group of 20. Data on $CL_R$ values of nicotine and cotinine and $CL_f$ of cotinine for the control group have not been previously published.

The $CL_R$ values of nicotine and cotinine were determined from the area under the plasma concentration–time curve (AUC) over the 8 hours after the test infusions and the urinary excretion over the same time period as follows:

$$CL_R = \frac{\text{Urine excretion}}{\text{AUC}}$$

($CL_{NR}$) was computed as follows:

$$CL - CL_R$$

The $CL_f$ of cotinine (i.e., the clearance of nicotine associated with the formation of cotinine) was computed as follows:

$$CL_f = CL_{NR,NIC} \times Fc$$

in which Fc is the fractional conversion of nicotine to cotinine.

As an in vivo probe for phenotypic cytochrome P450 2D6 activity, we administered 30 mg dextromethorphan to the index case subject at bedtime on the evening of the third day after the nicotine-cotinine infusion.[7] An overnight urine collection was obtained for measurement of dextromethorphan and its metabolite dextrorphan by HPLC.[8]

## RESULTS

The index case subject was a 57-year-old woman who enrolled in a smoking cessation treatment study (run by Dr. Sachs).[9] While smoking 20 cigarettes per day during the pre-quit baseline period, she had the expected plasma nicotine concentration of 23 to 32 ng/ml (mean, 27.9 ng/ml; measured on three occasions), but she had an unusually low plasma cotinine concentration of 33 to 38 ng/ml (mean, 35.0 ng/ml) as opposed to an expected value of about 300 ng/ml for that level of smoking. Likewise, while receiving treatment with a nicotine patch that delivered 15 mg every 16 hours and not smoking for 12 consecutive weeks, she had nicotine levels that averaged 17.9 ng/ml, whereas cotinine levels averaged 22.8 ng/ml (plasma cotinine to nicotine ratio, 1.2). In contrast, in 95 people undergoing smoking cessation treatment with nicotine patch therapy at the same clinic and in whom abstention from cigarette smoking was documented, the average ratio of plasma cotinine to plasma nicotine was 16.1 (95% confidence interval, 12.8 to 19.3).

The plasma concentrations of labeled nicotine and cotinine after intravenous infusion in the index case subject and in the control group of 20 smokers are shown in Fig. 2. In the control group, on average, 72% of nicotine was converted to cotinine (Table I). There were no significant differences in nicotine or cotinine kinetics between men and women. In the index case subject, blood levels of cotinine-$d_2$ derived from nicotine-$d_2$ were extremely low, whereas the disposition kinetics of the infused cotinine-$d_4$ were normal (Fig. 2 and Table I). The index case subject converted only 9% of nicotine to cotinine and had an unusually low total clearance and $CL_{NR}$ (metabolic) of nicotine, as well as a long half-life of nicotine, compared with the control subjects. The $CL_f$ of cotinine was markedly low in the index case subject. The kinetics of cotinine were similar in the case subject and the control subjects. The metabolic ratio of dextromethorphan to dextrorphan in the urine of this subject was 0.005, indicating that she is an extensive metabolizer of this substrate for CYP2D6.

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CLINICAL PHARMACOLOGY & THERAPEUTICS
VOLUME 57, NUMBER 5



## DISCUSSION

This report provides the first complete kinetic characterization of a patient with deficient C-oxidation of nicotine to cotinine. The nature of the enzymatic defect is unknown. Nicotine is known to be converted to cotinine in a two-step process involving cytochrome P450 and cytosolic aldehyde oxidase. In studies of nicotine metabolism by human microsomes in vitro, Cashman et al.[10] found that CYP2A6 was the main isozyme responsible for nicotine C-oxidation. Flammang et al.,[11] using a complementary deoxyribonucleic acid (cDNA)–directed expression system in human hepatoma cells, reported that other cytochrome P450 isozymes, including 2B6, 2C9, and 2E1, exhibited the greatest degree of metabolic activity for nicotine. McCracken et al.,[12] using a human β-lymphoblastoid cell cDNA expression system, found the greatest cotinine formation from nicotine for 2B6, 2D6, and 2A6. Cholerton et al.[13] recently reported five subjects in a group of 124 who had unusually high nicotine/cotinine ratios in the urine after each had taken a capsule of nicotine orally. These subjects presumably have relatively deficient C-oxidation of nicotine, although they could also have abnormal metabolism or excretion of cotinine. Of note is that these five subjects were found to be homozygous for CYP2D6 mutations and were phenotypically poor metabolizers of debrisoquin. On the other hand, not all deficient metabolizers of debrisoquin were poor metabolizers of nicotine. In contrast, our index case subject was a fast metabolizer of dextromethorphan, another probe drug for CYP2D6 activity.[7] Therefore, although the enzymatic defect responsible for deficient C-oxidation of nicotine in our subject has not been identified, we can exclude deficient CYP2D6 activity.

Deficient C-oxidation of nicotine appears to be associated with unusually slow elimination of nicotine. We have shown previously that the clearance of nicotine is positively correlated with the extent of conversion of nicotine to cotinine, indicating that the metabolism by way of cotinine is the most rapid pathway of nicotine metabolism.[6] That deficient generation of cotinine is associated with slow metabolism of nicotine is consistent with this proposition.

The prevalence of deficient C-oxidation of nicotine in the population is unknown, but it appears to be low. We have studied the metabolism of nicotine and cotinine in more than 150 individuals and have seen only one other similar case (Benowitz NL, et al. Unpublished data, 1995). Of the 220 subjects in the smoking treatment trial,[9] based on plasma nicotine and cotinine measurements during ad libitum smoking in the pre-quit baseline period, our index case subject was the only one who showed a very low cotinine to nicotine ratio. On the other hand, Cholerton et al.[13] reported five cases with high nicotine/cotinine ratios in 124 people. It is unclear from the Cholerton report whether the formation of cotinine was as low in these subjects as in our index case subject. Thus, based on the available data, the prevalence of deficient C-oxidation of nicotine appears to be somewhere between 0.5% and 4% in the general population.

The biological implications of deficient C-oxidation of nicotine are unclear but could be of great significance. Because smokers tend to regulate blood levels of nicotine from tobacco, slow metabolizers of nicotine might be expected to consume less tobacco smoke and therefore may be at a lesser risk for smoking-related disease. On the other hand, an unusually long half-life of nicotine would also mean that nicotine levels stay higher during the times that smokers are not smoking, including overnight, potentially leading to greater adaptive changes in brain nicotinic receptors and perhaps more severe physical dependence. In addition, cotinine may have pharmacologic activity that differs from that of nicotine but contributes to the addictive or other pharmacologic effects of nicotine.[5] Individuals with deficient C-oxidation, as in our index case subject, would therefore be expected to experience a lesser contribution of cotinine to the action of nicotine.

Finally, cotinine measured in plasma, saliva, or urine is widely used as a biomarker of nicotine intake from tobacco.[4] Cotinine levels might be used to stratify patients to likely levels of addiction and to predict optimal doses of nicotine replacement therapy (e.g., nicotine gum or transdermal nicotine). Individuals with deficient C-oxidation of nicotine may be incorrectly diagnosed as light smokers and be inappropriately treated. One way to detect these individuals would be to measure exhaled carbon monoxide or blood carboxyhemoglobin, as well as cotinine. If the carbon monoxide level is disproportionately high compared with the cotinine level, a plasma nicotine measurement is suggested. The plasma cotinine to nicotine ratio would ascertain whether the anomaly represents deficient conversion of nicotine to cotinine.

We thank Dr. Urbain Säwe (Kabi Pharmacia, Helsingborg, Sweden) for initially noting the unusual cotinine level and bringing it to our attention; Patricia Buley, Sandra Tinetti, and Lynne Rowe for assistance in clinical studies; Irving Fong and Dr. Thomas Delahunty for analytical chemistry; and Kaye Welch for editorial assistance.

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*References*

1. U.S. Department of Health and Human Services. The health consequences of smoking: nicotine addiction. A report of the Surgeon General. Washington, DC: Government Printing Office, 1988 (DHHS publication no (CDC) 88-8406):580.
2. Benowitz NL. Pharmacologic aspects of cigarette smoking and nicotine addiction. N Engl J Med 1988; 319:1318-30.
3. Benowitz NL, Jacob P III, Fong I, Gupta S. Nicotine metabolic profile in man: comparison of cigarette smoking and transdermal nicotine. J Pharmacol Exp Ther 1994;268:296-303.
4. Benowitz NL. The use of biologic fluid samples in assessing smoke consumption. In: Grabowski J, Bell CS, eds. Measurement in the analysis and treatment of smoking behavior, NIDA monograph 48. Washington, DC: Government Printing Office, 1984:6-26.
5. Keenan RM, Hatsukami DK, Pentel PR, Thompson T, Grillo MA. Pharmacodynamic effects of cotinine in abstinent cigarette smokers. CLIN PHARMACOL THER 1994; 55:581-90.
6. Benowitz NL, Jacob P III. Metabolism of nicotine to cotinine studied by a dual stable isotope method. CLIN PHARMACOL THER 1994;56:483-93.
7. Henthorn TK, Benitez J, Avram MJ, et al. Assessment of debrisoquin and dextromethorphan phenotyping tests by gaussian mixture distributions analysis. CLIN PHARMACOL THER 1989;45:328-33.
8. Park YH, Kullberg MP, Hinsvark ON. Quantitative determination of dextromethorphan and three metabolites in urine by reverse-phase high-performance liquid chromatography. J Pharm Sci 1984;73:24-9.
9. Sachs DPL, Säwe U, Leischow SJ. Effectiveness of a 16-hour transdermal nicotine patch in a medical practice setting, without intensive group counseling. Arch Intern Med 1993;153:1881-90.
10. Cashman JR, Park SB, Yang ZC, Wrighton SA, Jacob P III, Benowitz NL. Metabolism of nicotine by human liver microsomes: stereoselective formation of trans-nicotine-*N'*-oxide. Chem Res Toxicol 1992;5:639-46.
11. Flammang AM, Gelboin HV, Aoyama T, Gonzalez FJ, McCoy GD. Nicotine metabolism by cDNA-expressed human cytochrome P-450s. Biochem Arch 1992;8:1-8.
12. McCracken NW, Cholerton S, Idle JR. Cotinine formation by cDNA-expressed human cytochromes P450. Med Sci Res 1992;20:877-8.
13. Cholerton S, Arpanahi A, McCracken NW, et al. Poor metabolisers of nicotine and CYP2D6 polymorphism. Lancet 1994;343:62-3.



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